Anaerobic toxicity assay of plasticisers

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ABSTRACT

Plasticisers are commonly found in landfill leachate and accumulate in the environment. Some of them are known as disruptive endocrine compound. This manuscript assessed the toxicity of three common plasticisers, including Bis(2-Ethylhexyl)phthalate (DEHP), o-hydroxybiphenyl (HBP) and 2,6-di-tert-butyl-4-(dimethylaminomethyl) phenol (MAMP) on the methanogens during the anaerobic process. It was found that DEHP and MAMP did not impede methanogenesis up to 200 mg/L, but no additional methane could be obtained from their degradation. In contrast, HBP severely inhibited methanogens at 200 mg/L, but after acclimatisation it could be metabolised resulting in a 25 % increase in methane production compared to the control.

Keywords: Anaerobic toxicity assay; plasticiser; phthalate.

INTRODUCTION

Landfill is one the most widely employed methods for the disposal of municipal solid waste around the world. Landfill leachate can be defined as the rain water percolating through landfills and containing harmful substances for the environment. Plastics can accumulate in the landfill because of their non-biodegradability, and components from the plastic can leach out and be found in landfill leachate. These toxic components are used during the manufacturing of plastics to improve their processability and are called plasticisers. These plasticisers can also be found on industrial sites and some are known to accumulate in the environment due to human activities.

Common plasticisers are o-hydroxybiphenyl (HBP) and Bis(2-Ethylhexyl)phthalate (DEHP). HBP consists of two linked benzene rings and a phenolic hydroxyl group, and is on the list of chemicals recognized as carcinogens by the state of California. [1] It was found at a concentration of 23 µg/L in the effluent of our anaerobic membrane bioreactor treating simulated municipal solid waste, [2] but some authors have reported a concentration of 2 µg/L in actual landfill leachate. [3] DEHP is the most important phthalate, is produced on a massive scale due to its good plasticizing properties, and is known to be a disruptive endocrine compound and has carcinogenic and mutagenic effects. [4-5] DEHP was reported to be barely biodegradable under anaerobic conditions, [6] while other authors [7] found that it could not be removed by aeration, coagulation/sedimentation or biological treatment. Gavala et al. [5] showed that degradation of DEHP occurred in a digester treating primary sludge, but accumulation of high levels of DEHP (more than 60 mg/L) had a negative effect on DEHP removal rates as well as on the biogas production. O’ Connor et al. [8] showed that DEHP exhibited a relatively high toxicity to methanogenesis over 100 mg/L. It was found in our lab-scale anaerobic bioreactor effluent at a concentration of 1 mg/L, and its concentration decreased in the aerobic polishing step placed after the anaerobic bioreactor. [2] Jonsson et al. [9] found phthalic acids at concentrations of 50 mg/L in landfills and observed that the concentration decreased over time. Another example of a commonly found plasticiser is 2,6-di-tert-butyl-4-(dimethylaminomethyl) phenol (MAMP) which is an antioxidant and stabiliser used as an oxidation inhibitor in natural and synthetic elastomers, polyolefin plastics, resins, adhesives, petroleum oil and waxes. [10]

Little is known regarding their biodegradability in the environment and even less about their toxicity towards methanogens. Methanogens are known to be the most sensitive trophic group in the anaerobic process, and any inhibition of their metabolism could cause an anaerobic digester to fail. Anaerobic digestion is becoming widely used to treat waste, and it is therefore important to know at which concentration these plasticisers will become an issue. Because some plasticisers can accumulate in the environment, it is important to know if these could have an effect on methanogens during the anaerobic treatment of leachate. This is particularly relevant in the case where plastics or industrial wastes are present in the municipal solid waste landfill.

MATERIALS AND METHODS

Three plasticisers (Table 1) that were found to be recalcitrants by GC-MS in our lab-scale anaerobic process were tested to determine their biodegradability and their toxicity towards methanogens: [2] o-hydroxybiphenyl (HBP), Bis(2-Ethylhexyl)phthalate (DEHP) and 3,5-Di-tert-butyl-4-hydroxyphenyl propionic acid. Unfortunately, the latter was not commercially available and 2,6-di-tert-butyl-4-(dimethylaminomethyl) phenol (MAMP) was used instead because of a very similar structural formula. These plasticisers were purchased from Sigma-Aldrich (analytical grade).

Plasticiser concentrations of 200, 20, 2, 0.2, 0.02 and 0.002 mg/L were tested in duplicate for each plasticiser in order to determine which concentration would cause cessation of methanogenesis. A wide range of concentrations was tested in order to determine not only at which concentration they start to inhibit methanogenesis, but also determine if at high concentration such as 200 mg/L these can be metabolised and ultimately converted to methane. Forty mg of acetic acid was put in each 38 mL glass bottle to act as a carbon source for the methanogens. A total volume of 20 mL of inoculum, Owen et al.'s buffered biomedium, [11] and plasticiser was added to each bottle while flushing with CO_2/N_2 gas (30/70) and sealed off immediately after. [11] The inoculum (2 mL in each bottle) was taken from an active anaerobic digester treating landfill leachate; [12] its total suspended solids and volatile suspended solids content were 22.9 and 16.9 g/L, respectively. Two controls containing acetic acid, inoculum and biomedium were run in parallel. The glass bottles were incubated in an orbital shaker at 35°C. The biogas volumes were regularly measured using a wetted glass syringe and reported at atmospheric pressure and a temperature of 35°C. The composition of gas was determined using a Shimadzu GC-TCD fitted with a Porapak N column (1500×6.35 mm).

RESULTS AND DISCUSSION

Previous studies on landfill leachate have extensively identified the compounds present in leachate, but not much is known about the tolerance of methanogens towards these toxicants. Interference with the metabolism of methanogenic cultures can manifest itself in several different ways in these tests. If the test compound is extremely toxic, it may inactivate all the microorganisms responsible for at least one step in the metabolic sequence. In a slightly less severe situation, the test compound may totally or partially inhibit microbial metabolism. If the compound does not completely inhibit metabolism, some bacterial activity will continue, and the culture may eventually acclimate to the compound, allowing a return to the same specific metabolic rate as in the absence of toxicant. [7]

The ATA was also used to determine whether methane production was due to acetic acid only, or if the plasticiser was also biodegraded. The evidence of toxicity in an ATA may be either a decreased initial gas production or a lag phase before gas production begins. In either case, the toxic effects should diminish with time and the ultimate gas production will reflect the additional gas generated by utilization of the test compound. In the bottles with 200 mg/L of plasticiser the theoretical COD masses introduced were 11.2, 10.3 and 10.5 mg COD for MAMP, DEHP and HBP, respectively. Hence, if the compounds were biodegraded there should be circa 25 % additional methane production for the 200 mg/L sample (COD from the plasticiser in addition to the 40 mg of acetic acid). The methane produced in the control with 40 mg acetic acid was circa 13 mL which is approximately 80 % of the theoretical value of 0.395 mL at 35°C per g COD removed.

Figure 1 shows that MAMP was not toxic at concentrations below 20 mg/L. However, with 200 mg/L, there was partial inhibition as methane production was more sluggish until day 14 (about half the control on day 14) before it acclimated and then returned to the same specific rate as in the absence of the plasticiser on day 21. Thus our results showed that at concentration usually found in leachate (ppb to ppm levels) this plasticiser will not affect methanogenesis. No additional methane could be produced from MAMP.

No inhibitory effects were observed with DEHP (Fig. 2), but DEHP could not be metabolized to produce methane. DEHP has a high hydrophobicity (log K = 8.7) which make it "stick" to the biomass, however, this did not affect methanogenesis even at 200 mg/L which is in contradiction with Gavala et al. [5] and O'Connor et al. [8] who found severe toxicity at concentrations of 60 and 100 mg/L, respectively. This shows that landfill leachate containing significant amounts of this persistent plasticiser could still be treated with no sign of imbalance. Jonsson et al. [9] observed that the concentration of DEHP decreased over time during methanogenesis in a landfill. This indicated that DEHP can be degraded to phthalic acid directly or via its monoester, but our results showed that it cannot be ultimately converted to methane.

In contrast, HBP was found to be the most toxic of the three plasticisers tested (Fig. 3): a severe inhibition was observed at 200 mg/L and it took more than thirty days for the methanogens to acclimatise and metabolise acetic acid to produce methane gas. No additional methane was obtained with MAMP and DEHP because of the control and the 200 mg/L curves were very similar. In contrast, with 200 mg/L of HBP the methane production was 25 % greater than in the control. Thus, HBP was biodegraded and converted to methane, and the lag phase was an adaptation period necessary to co-metabolize acetic acid and HBP. This is in agreement with previous studies where HBP was found to be biodegraded by *Pseudomonas* species via successive steps. [13-14] Thus our results showed that at concentration usually found in leachate (ppb to ppm levels) these three plasticisers will not affect methanogenesis.

CONCLUSIONS

ATA results showed no toxicity effect at concentrations equal to and below 20 mg/L for MAMP. However, at 200 mg/L a 50 % drop in methane production was observed on day 14 after which it returned to the same levels as the control. No effects on methanogens were noticed for Bis(2-Ethylhexyl)phthalate. These two plasticisers may have been biodegraded but could not be converted to methane. Finally, for o-hydroxybiphenyl a 75 % drop in methane production was noticed at 20 mg/L on day 14, whereas no gas was produced at 200 mg/L before thirty days. After acclimation, o-hydroxybiphenyl at 200 mg/L could be biodegraded resulting in a 25 % increase in methane production compared to the control.

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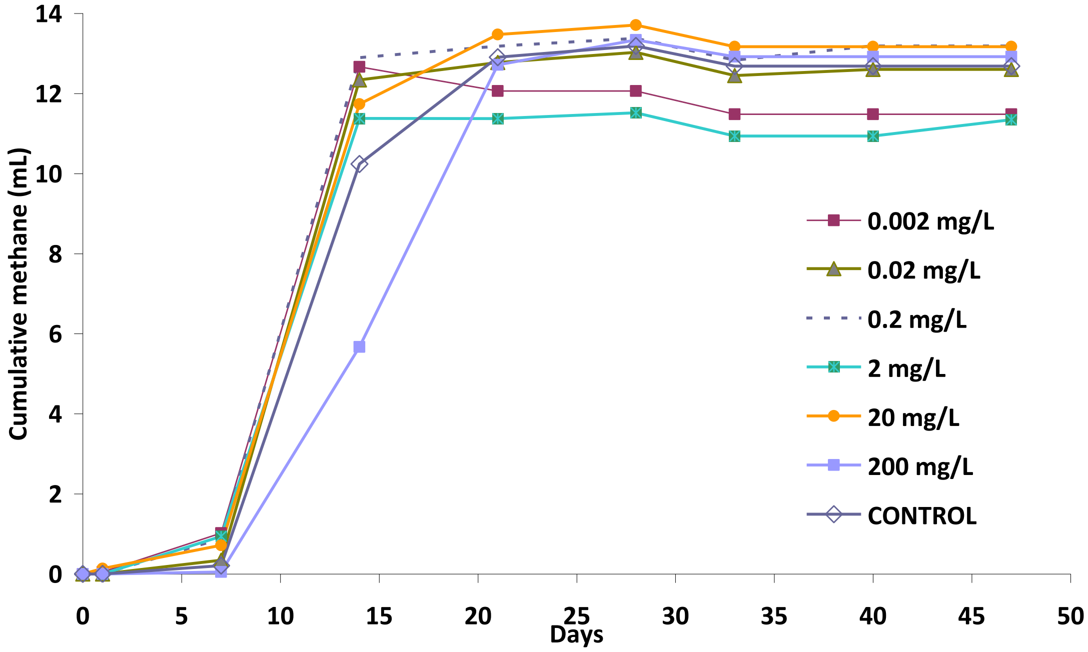
LIST OF FIGURE AND TABLE CAPTION

Figure 1. Anaerobic Toxicity Assay of 2,6-di-tert-butyl-4-(dimethylaminomethyl) phenol (MAMP). The maximum standard deviation was 0.2 mL.

Figure 2. Anaerobic Toxicity Assay of Bis(2-Ethylhexyl)phthalate (DEHP). The maximum standard deviation was 0.2 mL.

Figure 3. Anaerobic Toxicity Assay of o-hydroxybiphenyl (HBP). The maximum standard deviation was 0.2 mL.



Cumulative methane (mL)
14
12
10
8
6
4
2
0
0
5
10
15
20
25
30
35
40
45
50
Days
0.002 mg/L
0.02 mg/L
0.2 mg/L
2 mg/L
20 mg/L
200 mg/L
CONTROL

Fig. 1

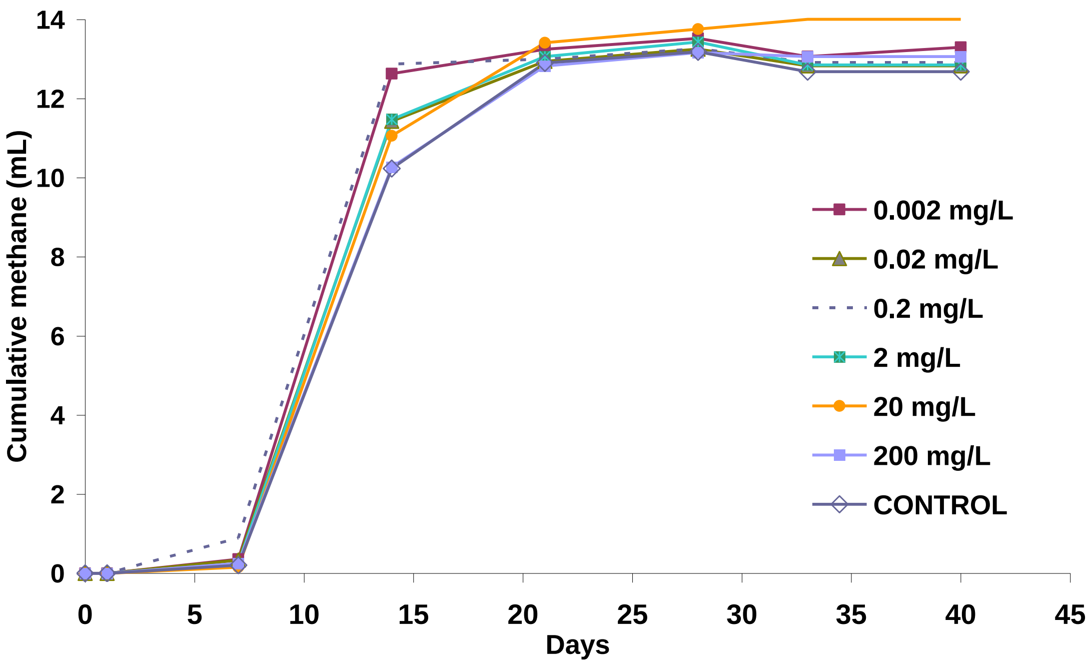

Cumulative methane (mL)
Days
0.002 mg/L
0.02 mg/L
0.2 mg/L
2 mg/L
20 mg/L
200 mg/L
CONTROL

Fig. 2

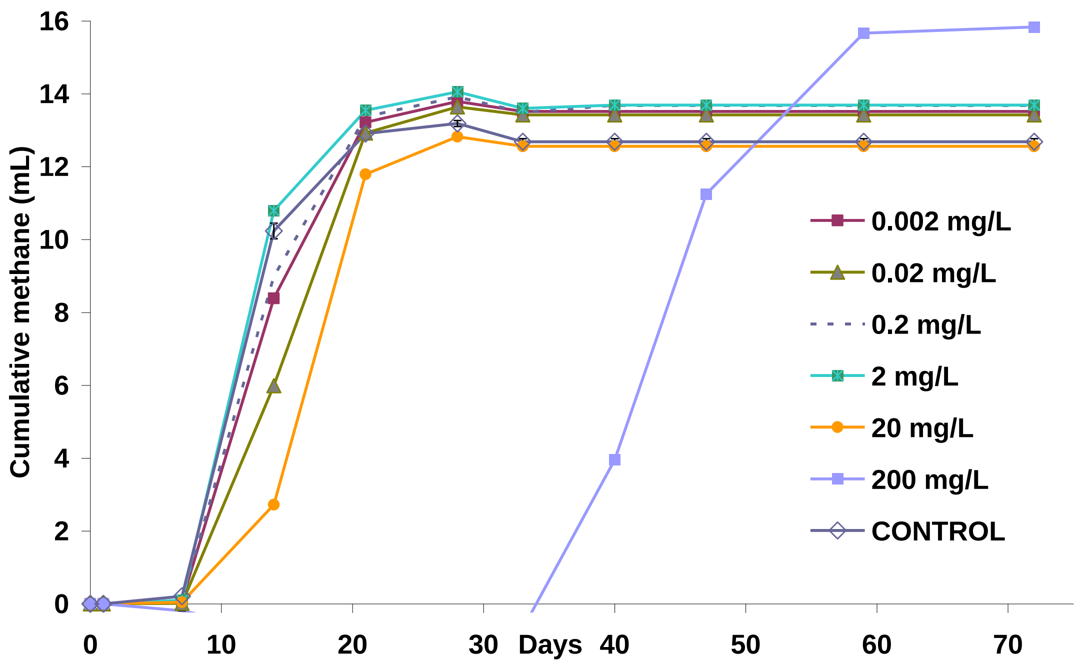
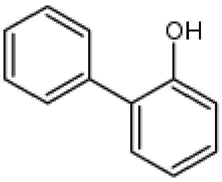
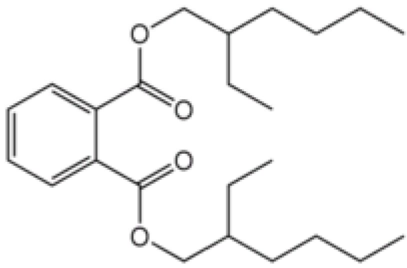
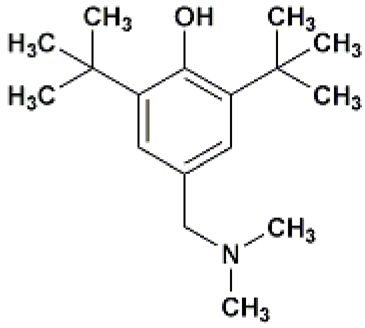

Cumulative methane (mL)
0
2
4
6
8
10
12
14
16
0
10
20
30
Days
40
50
60
70
0.002 mg/L
0.02 mg/L
0.2 mg/L
2 mg/L
20 mg/L
200 mg/L
CONTROL

Fig. 3

Table 1. Properties of the plasticisers used for the ATA test in this study. P = octanol/water partition coefficient. LD_{50} = median lethal dose for rats.

Plasticiser (acronym)	o-hydroxybiphenyl (HBP)	Bis(2-Ethylhexyl)phthalate (DEHP)	2,6-di-tert-butyl-4-(dimethylaminomethyl)phenol (MAMP)
Molecular formula	$C_{12}H_{10}O$	$C_{24}H_{38}O_4$	$C_{17}H_{29}NO$
Structural formula			
CAS number	90-43-7	117-81-7	88-27-7
Molecular weight	170.21	390.56	263.42
LD50 (mg/kg)	1050	1370	343
Density	1.293	0.98	0.95
Log P	2.94	8.7	4.6
Solubility (g/L)	0.26	10^{-4}	6.1 at pH 7